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Effects of the Herbicide 2,4-Dichlorophenyl(rho)-Nitrophenyl Ether (NIT) on Serum Thyroid Hormones in Adult Female Mice

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16. ABSTRACT

The administration of the herbicide 2,4-Dichlorophenyl-p-nitrophenyl ether (nitrofen; NIT) to adult female mice by i.p. injection at doses of 500 and 1000 mg/kg/day for 3 days, reduced serum thyroxine (T4) levels. T4 levels were reduced in the high-dosage group by 60% of the control and by 20% in the low-dose group. Serum triiodothyronine (T3) levels were relatively unaffected by NIT treatment at either dose. The animals appeared euthyoid, as body weights were unaffected even though T4 levels were reduced. Liver weights were slightly higher as a result of NIT exposure at both 500 and 1000 mg/kg/day.

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THE EFFECTS OF THE HERBICIDE 2,4-DICHLOROPHENYL-*p*-NITROPHENYL ETHER (NIT) ON SERUM THYROID HORMONES IN ADULT FEMALE MICE

(Thyroxine; triiodothyronine; nitrofen)

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SUMMARY

The administration of the herbicide 2,4-dichlorophenyl-*p*-nitrophenyl ether (nitrofen; NIT) to adult female mice by i.p. injection at doses of 500 and 1000 mg/kg/day for 3 days, reduced serum thyroxine (T4) levels. T4 levels were reduced in the high-dosage group by 60% of the control and by 20% in the low-dose group. Serum triiodothyronine (T3) levels were relatively unaffected by NIT treatment at either dose. The animals appeared euthyroid, as body weights were unaffected even though T4 levels were reduced. Liver weights were slightly higher as a result of NIT exposure at both 500 and 1000 mg/kg/day.

INTRODUCTION

NIT is considered to be a relatively nontoxic pre-emergence herbicide, having an $LD_{50} > 1$ g/kg [1]. Concern has arisen, however, over the safety of this compound because it is teratogenic in rodents at dose levels 2 to 3 orders of magnitude below the LD_{50} value [1–4]. During our studies of the teratogenic potential of NIT, it appeared that many of the effects of NIT were similar to the effects of chemicals that alter the hypothalamic-pituitary-thyroid axis during the perinatal period. In both instances growth rates are reduced and the lungs [3, 5], Harderian glands [2, 3, 6] and reproductive systems are underdeveloped. Other studies on the toxicity of NIT have reported results that also suggest an alteration of thyroid function. For example, Ambrose et al. [1] reported that chronic dietary exposure to NIT reduced

Abbreviations: DMSO, dimethylsulfoxide; PCB, polychlorinated biphenyl; NIT, 2,4-dichlorophenyl-*p*-nitrophenyl ether; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

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growth and National Cancer Institute bioassay study on NIT noted thyroid abnormalities in rats [7]. The similarity in structure between NIT and the thyroid hormones also suggests that NIT may have anti-thyroid and/or thyromimetic activity; they are all halogenated diphenyl ethers. As a result the present study was designed to directly test the hypothesis that exposure to NIT would produce an alteration of thyroid function in the adult female mouse.

METHODS

Nulliparous 90-day-old female mice (CD-1; Charles River) were housed in groups of five. 30 females were randomly selected and assigned to treatments in a manner that provided each treatment with mice with equal means and variances in body weights. The three dose levels of NIT were 0, 500 and 1000 mg/kg/day with 10 females at each dose. At 11.00 h females were dosed with NIT (91% pure) in DMSO (2.5 μl/g body weight) by i.p. injection for 3 consecutive days. After the last injection the females were necropsied between 13.30 and 15.30 h. The 30 mice were necropsied in 10 blocks, with each block containing 1 female from each of the three dosage groups to insure that treatment effects were not confounded with the time of day, the time interval since dosing and other nuisance variables that might influence T3 or T4 levels. The mice were anesthetized in CO_2 and blood was drawn by cardiac puncture in 6 ml serum separation tubes which contain a clot activator. After 60 min the blood samples were centrifuged for 15 min at 3.7 K (10 000 $\times$ g) and the serum was subsequently stored at $-20°C$. The serum hormone analyses were performed by Vetpath Laboratory, Teterboro, NJ using Corning's immunophase T4 and T3 radioimmunoassay test reagents. T4 levels are reported in μg/100 ml and T3 levels are in ng/100 ml. Body and liver weights were measured at this time. The data were analyzed on SAS [8] at EPA. The body weight at necropsy was used as a covariate and the linear dose responses were determined. Significant effects were determined using the partial sums of squares from these analyses. When the overall analysis was significant treatment means were compared using t-test and/or orthogonal contrasts for the planned comparisons.

RESULTS

The results of the present experiment are shown in Table I and the statistical analyses are shown in Table II. Body weight and weight change during the 3-day dosing period were unaffected by treatment. An analysis for block effects indicated that the order in which the females were necropsied did not effect serum T3 or T4 levels. Liver weights were slightly elevated in both dosage groups ($P < 0.05$, one-tailed t-test for both doses) being 11% heavier than the control value. Serum T3 levels were slightly lower in the high-dose group (81% of control, $P < 0.07$, one-tailed t-test). In contrast to the other parameters in the present study, serum T4

TABLE I

THE EFFECTS OF THREE DAILY i.p. INJECTIONS OF NIT ON BODY AND LIVER WEIGHTS AND SERUM T4 AND T3 LEVELS

Variable	Dose of NIT (mg/kg/day) for 3 days		
	0	500	1000
Pre-dose body weight (g)	28.3 (0.25), 10	27.7 (0.60), 10	28.9 (0.35), 10[a]
Final body weight (g)	27.9 (0.60), 10	27.7 (0.51), 10	27.9 (0.70), 9[b]
Weight change (g)	−0.52 (0.49)	−0.14 (0.43)	−0.91 (0.48)
Liver weight (mg)	1476 (54)	1631 (54)*	1644 (57)*
Serum T3 (ng/dl)	43.2 (3.3), 10	41.8 (3.3), 10	38.4 (3.5), 9
Serum T4 (mcg/dl)	2.15 (0.21), 8	1.67 (0.20), 9*	0.88 (0.25), 7**[c]

[a]Values are means (standard errors), numbers of mice.
[b]One female died during dosing.
[c]Sample sizes are less for T4 levels because there was an insufficient quantity of serum in some samples to measure both T3 and T4. *$P < 0.05$ (one-tailed t-test); **$P < 0.001$ (two-tailed t-test).

TABLE II

STATISTICAL ANALYSES OF THE EFFECTS OF NIT ON ADULT FEMALE MICE

Variable	DF	Final weight	Weight loss	Liver weight	Serum T3	Serum T4
Dose	2	NS	NS	NS	NS	[c]
Dose (linear)	1	NS	NS	[a]	NS	[c]
Covariate (final) body weight	1	–	[b]	[c]	[b]	NS
Orthogonal Contrast Con vs. NIT	1	NS	NS	[b]	NS	[b]

NS, not significant.
[a]$P < 0.10$ (two-tailed); [b]$P < 0.05$; [c]$P < 0.001$.

levels were drastically reduced by NIT treatment. Statistical analysis indicated that this effect was linear, $F(1,22) = 15.6$, $P < 0.001$. The serum T4 levels in mice from the 500 mg/kg/day group were 78% of control ($P < 0.05$, one-tailed t-test) and the 1000 mg/kg/day group was 41% of control ($P < 0.0005$, two-tailed t-test).

During the necropsy it was noticed that many coarse black granules of NIT were still present in the peritoneal cavity of the treated females adhering to the intestines and not absorbed. Therefore, the effective doses used in the present experiment are less than the doses that were administered.

DISCUSSION

Results of the present study clearly demonstrate that NIT alters thyroid function

in the adult female mouse. Serum T4 levels were drastically reduced to 40% of control values after three daily injections of NIT at 1000 mg/kg/day. While the T4 levels were suppressed by NIT treatment, serum T3 levels were relatively unaffected and body weights were normal, even in the highest dosage group. Similar effects on thyroid physiology and growth have been reported in studies with rats on low-iodine diets [9] and in rats fed PCBs at 250 ppm in the diet [10]. Rats in those studies also appeared euthyroid, and, like the mice in the present study, showed no changes in body weight. Similarly, in one of the few reports on the thyroid physiology of mice, Walker et al. [11] reported that mice receiving propylthiouracil in the drinking water (0.05% solution) had goiters which were five times as large as the control thyroid glands but body weights were unaffected. These studies demonstrate the robustness of homeostasis with respect to T3 levels (the active hormone) and growth and indicate that a prolonged suppression of the prohormone, thyroxine (T4), is required before growth rates will decline due to hypothyroidism. With this in mind, the NIT-induced growth retardation in rats fed NIT at 100 and 1000 ppm for 52 weeks [1] may represent a prolonged suppression of T4 production. Our reanalysis of the data presented in Table 2 of that paper indicates that growth was significantly ($P < 0.01$) retarded by NIT at 100 ppm and above, which translates to a dose of 10 to 15 mg/kg/day. It would seem that lethality is an inappropriate endpoint in the assessment of the toxicity of a chemical like NIT which alters endocrine function as this dose is more than two orders of magnitude below the LD_{50} value. Even complete thyroidectomy does not cause death in an adult rodent, although the animal's metabolism is severely altered [12]. In addition to the effects on serum T4 noted in the present study, a National Cancer Institute bioassay of NIT implicates it as an antithyroid compound. Rats fed NIT for their lifetime had a higher incidence of thyroid cysts and hyperplasia. 20 abnormalities were noted in 184 rats fed NIT as compared to 0 out of 36 controls [7].

In addition to the noted effects on the thyroid, NIT also alters liver function. Mice in the NCI study developed liver tumors as a result of NIT exposure [7] and chronic NIT exposure causes liver enlargement [1]. Dams exposed to NIT at doses of 50 mg/kg/day and above during gestation (days 7 to 17) had enlarged livers at term (Chernoff et al. in preparation), and the mice in the present study had slightly enlarged livers as compared to controls. It is possible that the effect of NIT on serum T4 levels is not entirely independent of the liver effect. Dietary exposure to PCBs lowers serum T4 levels by stimulating the metabolism of T4 by the liver by inducing T4-UDP-glucuronyltransferase and increasing biliary flow without affecting serum T3 levels [13]. Therefore, NIT may induce T4 metabolism and excretion which, in turn, enhances T4 turnover lowering serum T4 levels. Alternatively, if NIT has thyromimetic activity it would inhibit both TRH secretion from the hypothalamus and the secretion of TSH by the anterior pituitary, which would in turn reduce serum thyroxine levels. In addition to these two possible mechanisms for the effect of NIT on serum T4 levels it should be noted that other

mechanisms of action are known for antithyroid compounds.

Although the present study clearly demonstrates that NIT alters thyroid function in the adult mouse, we have not proven that this biological activity is responsible for any of the teratogenic effects noted in earlier studies. In these studies we found that prenatal NIT exposure caused many effects which were similar to perinatal hypothyroidism [2, 3]. Growth was permanently retarded, the Harderian glands were small or missing, the lungs were small and the reproductive development was delayed. All these effects can be mimicked by producing hypothyroidism in the neonatal rat [5, 6, 12]. Further studies are necessary to evaluate the merit of this hypothesis.

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